Human pulpal response to Mineral Trioxide Aggregate (MTA): A histologic study

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The purpose of this study was to study the histologic changes in the dental pulp following pulpotomy with Mineral Trioxide Aggregate (MTA) and Calcium hydroxide. Pulpotomies were performed on premolar teeth that were to be extracted for orthodontic reasons. The radicular pulp was capped with either MTA or Calcium hydroxide and restored with IRM. The teeth were extracted at 4 and 8 week intervals, fixed in 10% formalin and then kept in 5% nitric acid for 28 days for demineralization. Longitudinal sections were then prepared and viewed under light microscope. The pulps capped with MTA (at the end of 4 weeks and 8 weeks) showed dentin bridge formation which was more homogenous and continuous with the original dentin when compared to the pulps capped with calcium hydroxide. The pulpal inflammation was also less in the MTA group as compared to the calcium hydroxide group at the end of 4 and 8 weeks.

J Clin Pediatr Dent 30(3): 203-210, 2006

INTRODUCTION

Vital pulp therapy procedures such as pulp capping and pulpotomies are perhaps two of the most commonly performed procedures by clinicians in their daily practice. These procedures are of greater significance to the pediatric dentist as they have proven to be highly successful in treating carious, mechanical and traumatic exposures in young permanent teeth. Although both these procedures have proven to be successful when judged clinically and radiographically, histologic examinations often show the evidence of chronic inflammation especially under carious exposures.

It was in 1920, with the introduction of calcium hydroxide by Hermann that a new era in vital pulp therapy began. Despite the numerous advantages of Calcium Hydroxide as a pulp capping agent, many authors still question the long term efficacy of using commercially available Calcium Hydroxide products for vital pulp therapy procedures.[1]

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In more recent times with the introduction of materials which are not only bio compatible but also bioinductive, the emphasis has shifted from mere preservation to regeneration. One such material which has shown immense potential for regeneration is Mineral Trioxide Aggregate (MTA). MTA was first developed and reported in the year 1993 by Lee, Monsef and Torabinejad. It is essentially a modified form of Portland cement composed of Calcium Silicate, Bismuth Oxide, Calcium Carbonate and Calcium Aluminate. When mixed with water, MTA forms crystals of Calcium Oxide in an amorphous structure of 33% Ca^{2+}, 49% PO_4^{3-}, 2% C, 3% Cl^- and 6 % Si^{2+}.[2] Although MTA was developed with the purpose of serving as a root end filling material, it has also proven to be successful in vital pulp therapy procedures both in animal[3] and human trials.[4] MTA is a biocompatible material and its sealing ability is better than that of amalgam or zinc oxide eugenol. It is a powder that sets in the presence moisture and has a pH of 12.5. It has a setting time of 4 hours and a compressive strength of 70 Mpa, which is comparable to IRM.[5] MTA also has the ability to stimulate cytokine release from bone cells indicating that it actively promotes hard tissue formation.[6] It was used experimentally for a number of years before it was approved for human usage by the U.S. Food and Drug Administration in the year 1998.[6] It is likely that MTA along with recombinant human bone morphogenic proteins (BMPs) will perhaps replace calcium hydroxide as "the material" for vital pulp therapy procedures in the future.

This study was undertaken to analyze the histologi-

cal changes in the dental pulp following pulpotomy with MTA and Calcium Hydroxide in order to establish a biologic basis for their use as pulpotomy medication.

MATERIALS AND METHOD

Patient Selection: Children in the age group of 11-14 years, scheduled to have their first premolars extracted for orthodontic treatment were included in the study. Ethical clearance and informed parent consent were obtained before proceeding with the study. The study included 31 non carious permanent premolar teeth from 10 patients (6 males and 4 females). The selected cases were divided into 2 groups:

GROUP A: In which pulpotomy was carried out and the radicular pulp was capped with calcium hydroxide. This group included a total of 15 teeth of which 8 were included in the 4 week (GROUP A_1) and 7 in the 8 week period (GROUP A_2) groups respectively.

GROUP B: In which, following pulpotomy, MTA was used to cap the remaining pulp. This group included a total of 16 teeth, i.e. 8 in the 4 week period (GROUP B_1) and 8 in the 8 week period (group B_2) respectively.

The teeth to be treated were an aesthetized with 2% xylocaine with epinephrin (1:200,000) (Astra Zeneca Pharma India LTD) by infiltration for maxillary premolars or block injections for mandibular premolars. After ascertaining that the teeth have been anesthetized, teeth were isolated, and access was obtained on the occlusal surface of teeth using a high speed hand piece with water spray with a number 4 round bur.

The roof of the pulp chamber was removed and coronal pulp tissue was scooped using a spoon excavator. The chamber was washed with saline to clear off the debris and bleeding was controlled with moist sterile cotton pellets. Once hemostasis was obtained, pulp was covered with either MTA or Ca $(OH)_2$. The Ca $(OH)_2$ (Deepti Dental Products India) was mixed according to the manufacturer's directions and applied to the exposure site, the remainder of the cavity was sealed with IRM (Dentsply, Caulk). MTA (Dentsply, Tulsa Dental) was mixed according to manufacturer's instructions and a 1 to 2 mm thick layer of freshly mixed MTA was placed over the exposed pulp. Then a moist flattened cotton pellet was placed over the MTA. The cotton pellet provided the moisture MTA required for the proper setting.[7] The remainder of the cavity was sealed with IRM.

At 4 week and 8 week intervals teeth from each experimental group were extracted. The extracted teeth were then kept in 10%formalin for 5 days then in 5% nitric acid for 28 days. Longitudinal sections were then prepared, stained with hematoxylene and eosin and viewed under light microscope.

HISTOLOGIC EXAMINATION

The sections were graded according to the criteria that were based on a modified scoring system adapted from Stanley as indicated in tables 1-8.[8]

Table 1. Pulpal inflammation

Score	Description
1	No inflammation
2	Minimal inflammation
3	Moderate inflammation
4	Severe inflammation
5	Abscess formation
6	Tissue necrosis

Table 2. Tissue reaction to the material

Score	Description
0	No Macrophages /giant cells adjacent to the material
1	Mild infiltration of macrophages/giant cells
2	Moderate infiltration of Macrophages/giant cells
3	Severe infiltration of Macrophages/giant cells

Table 3. Location of Dentin Bridge

Score	Description
1	At the interface of exposure pulp
2	Not at the interface of exposure pulp
3	Combination

Table 4. Impaction of capping agent

Score	Description
1	No impaction of pulp capping agent
2	Impaction of pulp capping agent

Table 5. Presence of dentin chips

Score	Description
0	No chips
1	Chipits
2	Double dentin bridges
3	Pulp stones

Table 6. Dentin bridge formation

Score	Description
0	No presence of bridge formation
1	Bridge formation < 25%
2	25% bridge formation < 50%
3	50% bridge formation < 75%
4	Bridge formation 75%

STATISTICAL ANALYSIS

The Mann-Whitney U test is used to test whether there is a significant difference between the average values of the two groups.

OBSERVATIONS AND RESULTS

On grading the sections according to the criteria that were based on a modified scoring system adapted from Stanley, the following scores and observations were observed.

On Statistical Analysis, a statistically significant difference was found between the 2 groups with respect to:

1) Inflammatory response: 6 out of the 8 samples in the CH group had a score 3 indicating moderate inflammation while 2 had a score 4 indicating severe inflammation; while in the MTA group 6 out of 8 samples had a score 1 indicating no inflammation while 2 had a score of 2 indicating minimal inflammation (p value of 0.000).
2) Quality of dentin: All 8 samples in the CH group had a score 1 indicating irregular pattern of tubules while all 8 samples in the MTA group had a score 2 indicating a regular pattern of tubules. (p=0.000).
3) Dentin bridge formation: 6 of the 8 samples in the CH group had a score 1 indicating bridge formation less than 25% while 2 samples had a score of 2 indicating 25-50% bridge formation. 6 of the 8 samples in the MTA group had a score 3 indicating a bridge formation of 50 – 75% while 2 samples had a score of 2 indicating 25-50% bridge formation.(with p=0.001).

Table 7: Quality of dentin formation in the bridges

Score	Description
0	No tubules present
1	Irregular pattern of tubules
2	Regular pattern of tubules

Table 8. Connective tissue in the bridge

Score	Description
0	No connective tissue
1	C.T. <25%
2	25% C.T. < 50%
3	50% CT < 75 %
4	CT – 75%

On Statistical Analysis, a statistically significant difference was found between the 2 groups with respect to:

1) Inflammatory response: 6 of the 7 samples in the CH group had a score 3 indicating moderate inflammation, while 1 had a score of 4 indicating severe inflammation. In the MTA group 6 of the 8 samples had a score 1 indicating no inflammation, while 2 showed minimal inflammation.(score2) (with a p value of 0.001.)
2) Quality of dentin: In the CH group all the samples had irregular pattern of tubules (score =1); while in the MTA group all showed irregular pattern of tubules. (Score =2) with p=0.000.
3) Dentin bridge formation: 5 of the 7 samples in the

Table 9. 4 WEEK PERIOD

Time –4 wks	Material	Pulpal inflammation						Tissue reaction				Impaction of Particles		Location of dentin bridge		
Score		1	2	3	4	5	6	0	1	2	3	1	2	1	2	3
GROUP A_1	CH			6	2			6	2			8		8		
GROUP B_1	MTA	6	2					7	1			8		8		

		Presence of Dentin Chips				Dentin bridge formation					Quality of Dentin Bridge			Connective tissue in the bridge				
Score		0	1	2	3	0	1	2	3	4	0	1	2	0	1	2	3	4
GROUP A_1	CH	2	6				6	2				8			8			
GROUP B_1	MTA	1	7					2	6				8		8			

Table 10. 8 WEEK PERIOD

Time –8 wks	Material	Pulpal inflammation						Tissue reaction				Impaction of Particles		Location of dentin bridge		
Score		1	2	3	4	5	6	0	1	2	3	1	2	1	2	3
GROUP A_2	CH			6	1			5	2			7		7		
GROUP B_2	MTA	6	2					8				6	2	8		

		Presence of Dentin Chips				Dentin bridge formation					Quality of Dentin Bridge			Connective tissue in the bridge				
Score		0	1	2	3	0	1	2	3	4	0	1	2	0	1	2	3	4
GROUP A_2	CH	5	2				2	5				7			6	1		
GROUP B_2	MTA	8						2		6			8		5	3		

CH group had a maximum score of 2 indicating a bridge formation of 25-50% while in the MTA group 6 of the 8 samples had a maximum score of 4 indicating bridge formation of greater than 75%, with p=0.004

DISCUSSION

Pulpotomy procedures are often performed when a pulpal exposure occurs in a young permanent tooth, whether the exposure is due to caries or to trauma. Because of an abundance of precursor cells and rich blood supply in young permanent teeth, pulp exposures as a result of caries or trauma has the capacity to heal through cell reorganization and bridge formation as long as an hermetic seal is maintained and bacterial microleakage is prevented. $Ca(OH)_2$ has remained the material of choice for vital pulp therapy procedures ever since Hermann demonstrated its ability to induce dentin bridging over an exposed pulp in the year 1920. Calcium Hydroxide has been shown to stimulate a rapid differentiation of odontoblast and odontoblast like cells that form a hard tissue barrier in the pulp.[9] Although Ca^{2+} ions from the $Ca(OH)_2$ are not incorporated into the mineralized tissue, it acts as a substrate for repair. The mineralization process is also aided by the alkaline nature of $Ca(OH)_2$ (pH=10.2). Thus it acts as a local buffer against the acidic reactions produced by the inflammatory process and also neutralizes lactic acid produced by the osteoclasts thereby preventing further destruction of the mineralized tissue. The alkaline pH also aids in activation of alkaline phosphatase activity which plays an important role in mineralization. $Ca(OH)_2$ has also got excellent antibacterial properties which are also attributed to it's alkaline pH. Most bacteria are destroyed at a pH of 9.5 and few can survive at pH 11 and above.[10]

Carlile MJ et al (2000) and Alliot – Licht B et al (2001) based on evidence from cultured human pulpal cells have suggested that the progenitor cells for the new-odontoblast like cells could be the pericytes[11,12] or the pericyte progenitor cells.[12,13] It is also possible that fibroblasts may redifferentiate as odontoblasts. Fitzgerald (1991) described cellular reorganization in exposed monkey pulp capped with a hard set calcium hydroxide. He also observed that wound healing occurred in a similar fashion to that seen in other connective tissue.[14] Growth factors which are present in the dentinal matrix also play a role in the differentiation and migration of odontoblast like cells required for dentinal bridge formation.[15,16] One subclass of these molecules, the "transforming growth factor ß (TGF- ß)" family appears responsible for signaling odontoblast differentiation and stimulating the odontoblasts to secrete tertiary dentin.[17] The factors are released during the demineralization of dentin associated with the progressing caries lesion. Greater amounts of growth factors have been found in tertiary dentin than in primary dentin and they contribute to dentinal matrix formation. Despite the numerous advantages of calcium hydroxide, one of the biggest disadvantages is the appearance of tunnel defects in the bridges following capping procedures and thus fails to provide an hermetic seal. McComb (1983)[18] and Hwas and Sandrik (1984)[19] have reported the softening and disintegration of $Ca(OH)_2$ products after 6 months leaving a void underneath the restoration and thereby a pathway for bacterial infection. More recently Cox et al (1996), in a study on primates observed that 89% of all dentin bridges had tunnel defects and 41% of the dentin bridges were associated with recurring pulpal inflammation.[20]

For vital pulp therapy procedures to be effective on a long term basis the development of materials which are biocompatible, bactericidal, bio-inductive and having good sealing ability is of primary importance. This has led to the experimentation with acid etched dentin bonding agents and MTA. Cox et al (1982) demonstrated that healing of the dental pulp is not exclusively dependant on the supposed stimulatory effect of a particular type of medicament but is directly related to the capacity of both the capping and definitive restorative material to provide a biological seal against immediate and long term microleakage along the entire restoration interface.[21]

Various animal[22,23] and human clinical trials[24,25] have

PULPAL INFLAMMATION—4 WEEKS

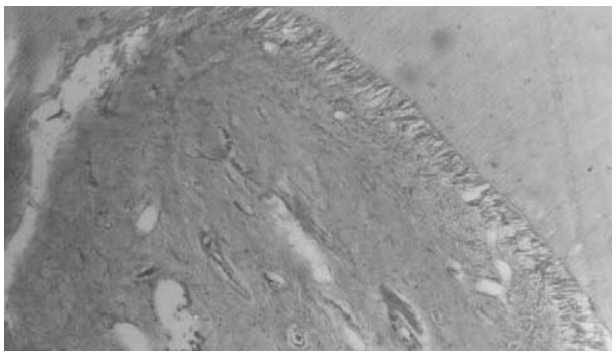

Figure 1. No Pulpal Inflammation in pulp capped with MTA

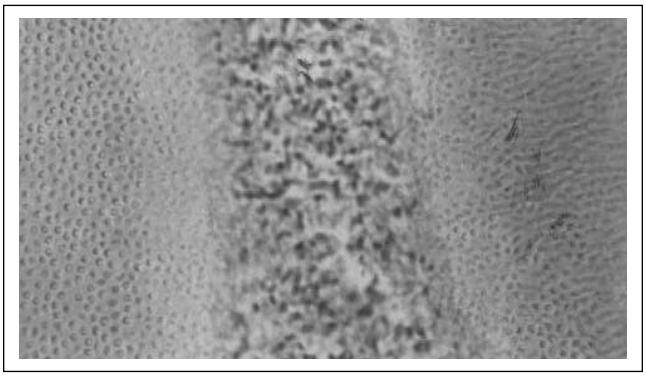

Figure 2. Chronic Inflammatory cell infiltration in Pulp capped with $Ca(OH)_2$

DENTIN BRIDGE FORMATION

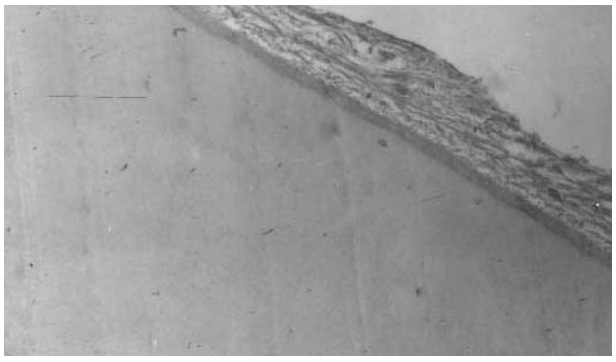

Figure 3. Dentin Bridge Formation over Pulp capped with MTA

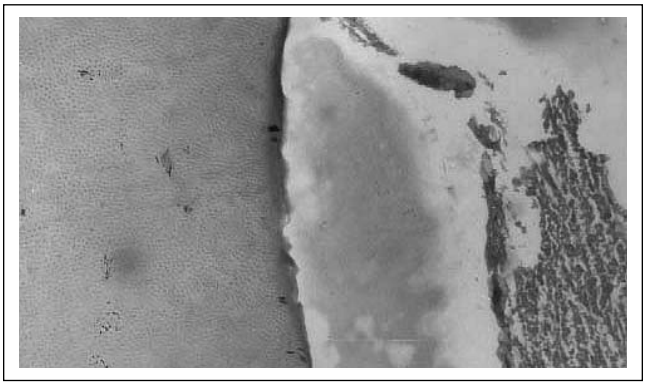

Figure 4. Dentin Bridge Formation over Pulp capped with $Ca(OH)_2$

PULPAL INFLAMMATION—8 WEEKS

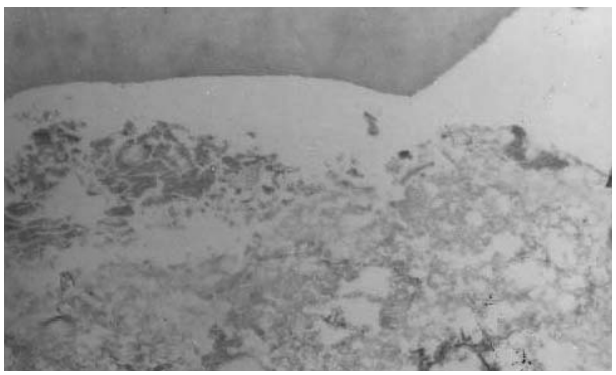

Figure 5. Pulpal inflammation in pulp capped with $Ca(OH)_2$

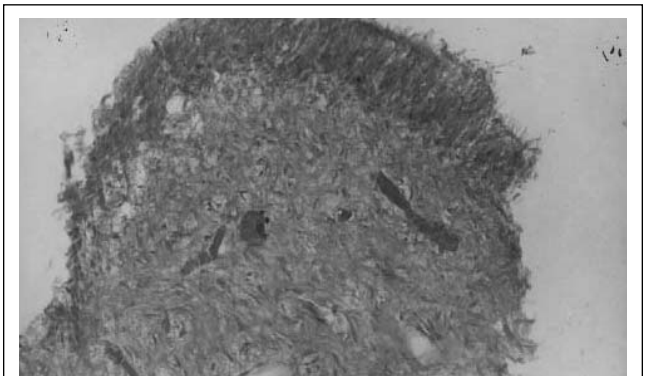

Figure 6. Minimal inflammation in pulp capped with MTA

DENTIN BRIDGE FORMATION—8 WEEKS

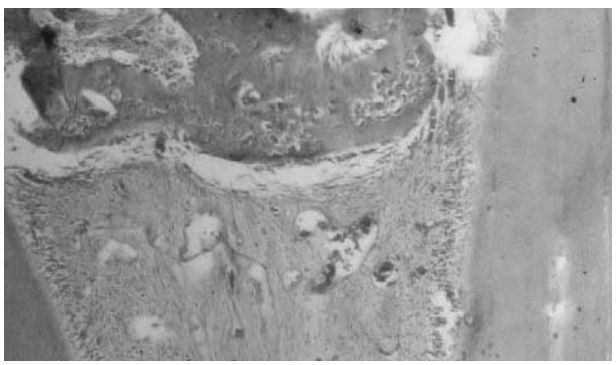

Figure 7. Dentin bridge formation with $Ca(OH)_2$

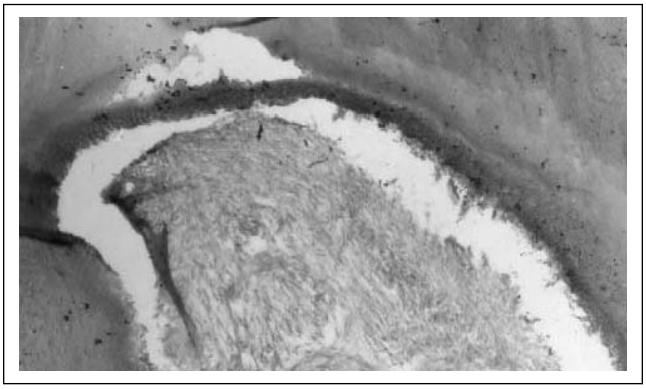

Figure 8. Dentin bridge formationn over pulp capped with MTA

shown a favorable response of pulpal tissue to MTA. In our study we used MTA (Pro Root supplied by Dentsply Tulsa Dental). Histopathologic examination of Group A (control group / $Ca(OH)_2$ group) and Group B (Experimental group / MTA group) were done at the end of 30 and 60 days. On grading the sections according to the criteria that were based on a modified scoring system adapted from Stanley, at the end of 30 days a statistically significant difference between the 2 groups was found with respect to pulpal inflammation, dentin bridge formation and quality of dentin.

Thus we find that MTA caused either very little or no inflammation regardless of the time period as compared to the severe inflammation caused by the $Ca(OH)_2$ group. The impaction of the pulpotomy agent into the pulp was not a discriminating factor because the findings were similar in both the groups. The impaction of the agent could be related to a combination of material composition and technique used. Preferably the material should be placed carefully on the exposed pulp surface and not pressed into the pulp tissue. Deep impaction of the material can decrease the rate of healing and bridge formation. Dentin chips were present in all the slides. They promote healing if confined to the superficial portions of the pulp but if they are numerous and localized deeper in the pulp tissue they may have a deleterious effect.[22] Both the groups showed bridging, mostly at the interface but MTA had more complete bridging regardless of the time frame. The presence of connective tissue in the bridges indicates that the bridges are not yet completely mineralized. This can be explained by the fast initial disorganized formation of reparative dentin that engulfs cellular inclusions. With time, the reparative dentin becomes more mineralized at the surface and more regular as the bridge matures and begins tubular dentin formation.

Our data demonstrating the favorable response of pulpal tissue to MTA following pulpotomy is similar to the observations and results reported by various authors including Dominguez et al (2003 in mongrel dogs),[22] Pittford et al (1996 in monkeys),[26] Aeinehchi M et al (2003).[24]

The favorable response of pulpal tissue to MTA following pulpotomy can be attributed to its physical properties. Besides being less cytotoxic than IRM/super EBA, having a compressive strength equal to some of the fortified Zinc Oxide eugenol bases (67.3Mpa at end of 21 days) it was also found to have good sealing ability. In fact leakage was less than amalgam/super EBA and is not affected by blood. MTA was found to set slowly with a mean setting time of 2 hrs 45 minutes, where as most of the traditional dental cements set much faster. The slower setting of MTA in turn leads to much lesser setting shrinkage as compared to the traditional dental cements and this could be one of the reasons for the reduced leakage seen with MTA. MTA had a pH of 10.2 after mixing which rose to 12.5 at 3 hours after which it remained constant. The alkaline pH could be the reason for its antibacterial properties. MTA was also found to stimulate cytokine release and its production and thus it was found to actively promote hard tissue formation rather than being inert like many dental materials.[8]

The mechanism of action of MTA in bridging could be similar to that of $Ca(OH)_2$. Calcium hydroxide has a direct effect on the precapillary sphincters resulting in less plasma outflow, which in turn favors a calcific response in the involved tissue.[27] Calcium hydroxide also increases the action of pyrophosphatase enzyme which is Ca^{2+} dependent. This enzyme transforms pyrophosphatase into orthophosphatase which increases energy utilization and therefore favors a defense mechanism. Holland *et al* has suggested that hard tissue deposition could be due to the CaO present in MTA which may have a similar mechanism of action to calcium hydroxide.[28] MTA also has lower values of Mg^{2+} which is speculated to slow down the mineralization process.[22] However, it should be remembered that the concept of bridging is a controversial issue, because the presence of a bridge does not necessarily imply that the pulp tissue is healthy. It can be viewed as both a healing response of a reaction to irritation.[29] Furthermore the formation of a bridge does not imply that the pulp will be sealed completely from the environment. The bridge formed is initially permeable but as time progresses, permeability decreases. Various investigators (Mjor – 1972[30] and Woehrlen – 1977[31]) have suggested that it is not always possible to section perfectly along the perpendicular axis of the tooth, therefore it is difficult to score all the sections and this is a major limitation of a study such as this. Also histologic demonstration of only one section through a dentin bridge following capping of an exposed pulp is not by itself a proper criterion for maintenance of long term pulpal healing. A long term study with a larger sample size is necessary to conclusively prove the efficacy of MTA in inducing a favorable pulpal response.

CONCLUSIONS

We can conclude that MTA gives a more predictable and positive response in pulpotomy procedures as compared to Calcium hydroxide. However longer term studies with a larger sample size and studies on cariously exposed teeth would further help to conclusively prove the efficacy of MTA in inducing a favorable pulpal response.

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